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## Asian Pacific Journal of Tropical Medicine

journal homepage: <http://ees.elsevier.com/apjtm>Original research <http://dx.doi.org/10.1016/j.apjtm.2016.03.034>Anti-*Toxoplasma* antibodies prevalence and associated risk factors among HIV patientsDechassa Tegegne<sup>1\*</sup>, Mukarim Abdurahaman<sup>1</sup>, Tadesse Mosissa<sup>2</sup>, Moti Yohannes<sup>1</sup><sup>1</sup>Department of Microbiology and Veterinary Public Health, Jimma University, P.O. Box 307, Jimma, Ethiopia<sup>2</sup>Department of Natural Resource Management, Jimma University, P.O. Box 307, Jimma, Ethiopia

## ARTICLE INFO

## Article history:

Received 15 Jan 2016

Received in revised form 16 Feb 2016

Accepted 15 Mar 2016

Available online 23 Mar 2016

## Keywords:

*Toxoplasma gondii*

HIV

LAT

Seroprevalence

## ABSTRACT

**Objectives:** To assess the seroprevalence and associated risk factors of toxoplasmosis among HIV patients in Agaro Town Health Center of Jimma zone.**Methods:** Convenient sampling was used to collect blood samples from 135 patients attending anti-retroviral therapy from February to March 2015. Serum samples were tested for anti-*Toxoplasma gondii* (*T. gondii*) antibody by using latex agglutination test. Structured questionnaire was used to collect data on socio-demographic and risk factors associated with toxoplasmosis.**Results:** Overall seroprevalence of toxoplasmosis was 80.7% (109/135, *CI*: 74.04–87.36). In multivariate analysis significant association was observed between anti-*T. gondii* seropositivity and raw meat consumption (*OR*: 3.514, *CI*: 1.167–10.581, *P* = 0.025), knowledge about toxoplasmosis (*OR*: 5.225, *CI*: 1.382, *P* = 0.015) and sex (*OR*: 4.023, *CI*: 1.382–19.762, *P* = 0.015).**Conclusions:** Immuno compromised patients showed high rate of seropositivity and thus, it is highly advisable to introduce routine anti-*T. gondii* antibodies serological screening test prior to ART commencement.

## 1. Introduction

*Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan parasite that can cause toxoplasmosis in animals and humans [1]. Feline species is the definitive host and plays a central role in the epidemiology of *T. gondii* infections by shedding resistant oocysts to the environment and thus serving as a significant source of infection for food animals and humans [2]. It has been estimated that one-third of the world human population is infected with toxoplasmosis and the incidence rate of 400–4000 congenital toxoplasmosis cases per year has been reported [3]. The principal route of acquisition of infections are raw or under cooked meat, vegetable or other feed or water, soil contaminated with oocyst and vertical transmission [4]. Among these sources of infection, raw or under cooked meat consumption has been reported to account for more than 30%–60% [5]. Toxoplasmosis has been considered as a major public health problem among immune-

compromised and pregnant women. It has been reported that the prevalence is higher in warm and humid environment [6].

Pregnant women with acute infection during pregnancy are at risk of exposing the unborn to congenital infection [7]. Wide geographic variation in the prevalence of latent *Toxoplasma* infection have been reported across the world in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa 30%–75% [8]. Geographical variation of prevalence of toxoplasmosis has been reported in different parts of the world: in United State 15% of childbearing age of women are infected with *T. gondii* [9], 44% in pregnant women in France [10], greater than 60% in Indonesia [8] and less than 8.38% in Eastern China [11].

Literatures indicate that *T. gondii* are asymptomatic in immune-competent persons (Studenicova *et al.*, 2006) but in immune-compromised individuals as CD4<sup>+</sup> T cell lymphocyte count decrease, reactivation of the latent *T. gondii* infection follows causing life threatening disease known as toxoplasmic encephalitis [12–14]. Prevalence rate of *Toxoplasma* infection among HIV positive individuals across the world has been found to vary from 3% to 97% [15,16]. According to the report of UNAIDS, 2011 Ethiopia ranks higher in HIV/AIDS prevalence and about 1.5 million people are infected and live with the virus. The seroprevalence of toxoplasmosis among HIV individuals

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Peer review under responsibility of Hainan Medical College.

Foundation project: The study was funded by Jimma University, College of Agriculture and Veterinary Medicine (No. 6223).

documented in different parts of Ethiopia include 88.2% in Arba Minch Hospital [16], 87.4% in Bahir Dar [17] and 60% in South West Ethiopia Mettu Karl hospital [18].

Routine monitoring of *T. gondii* antibodies has been gaining great importance in immune-compromised individuals though it is not a routine practice in different health care centers of Ethiopia. Even the existing majority of seroprevalence studies are limited to HIV/AIDS patients alone with little emphasis to associated risk factors. Furthermore, there has been no document of toxoplasmosis seroprevalence study in Agaro town. Therefore, the objective of this study was to detect the level of prevalence of latent toxoplasmosis in HIV positive patients registered and under follow-up by health professionals. In addition, it was to assess socio-demographic risk factors associated with this infection and forward recommendations that will improve health service delivery.

## 2. Materials and methods

### 2.1. Study design and area

The study design was cross-sectional based in public health center and convenient sampling was used based on inclusion criteria of patient's willingness to participate in the study from list of record book of the health center attending anti-retroviral therapy (ART) at Agaro town health center from February to March 2015. Agaro town is located in south western Ethiopia, in Oromia National Regional State, Jimma zone, Agaro Woreda, at a distance of 396 km from Addis Ababa. Its astronomical location is 7° 49' North Latitude and 36° East Longitude at an elevation ranging from 880 m to 3360 m above seas level (source Agaro city administration). The area receives mean annual rainfall of about 1530 mm that comes from the long and short rainy season and the mean annual temperature ranges from 25 °C to 30 °C. Agaro town has a total population of 28273.

### 2.2. Study population and sample size

The study populations were human immunodeficiency virus (HIV) positive patients who were on follow up at Agaro town health center for ART. Presently 706 HIV (457 females and 249 males) positive patients attending anti-retroviral therapy. The total sample size was 135; calculated using Thrusfield [19] formula based on prevalence of 88.2% taken from a previous study conducted in Arba Minch Hospital [16], 0.05 margin of error (d) and at confidence level of 95%. Accordingly, one month data of patients visiting the health center for therapy and counseling was used to recruit participants of this study.

### 2.3. Data collection

About 5 mL of venous blood without anticoagulant was aseptically collected by trained medical laboratory technician following standard procedure from 135 HIV positive patients. Serum was separated and stored at -20 °C until the analyses was conducted at Microbiology laboratory of Jimma University. Trained ART attendant nurse interviewed the participants using structured and pretested questionnaire to collect socio-demographic characteristics and risk factors associated with *T. gondii* infection.

### 2.4. Serological method

A rapid slide agglutination Toxo-latex test (SPINREACT, S.A/S.A.U Ctra Santa Coloma, Spain) was used strictly following manufacturer instructions for qualitative and semi-quantitative detection of anti-*Toxoplasma* antibodies with analytical sensitivity of 4 IU/mL. Briefly, the test contains latex particles coated with soluble *T. gondii* antigen that are agglutinated when mixed with serum samples containing antibodies against *Toxoplasma* infection. In all sample analysis conducted both positive and negative control tests were performed to monitor the performance of the procedures and technical procedure was carried out correctly. The kit has diagnostic sensitivity and specificity of 96.1% and 89.6%, respectively.

### 2.5. Data management and analysis

Data was recorded and coded in excel spread sheet before analysis using statistical software SPSS version 22. Descriptive statistics was used to summarize the data and hence, the prevalence of toxoplasmosis was calculated as the number in study population testing positive to serological test divided by the total study unit tested. A logistic regression model was used to check the association of the disease toxoplasmosis with potential risk factors. Non-collinear variables that presented  $P < 0.05$  in univariable analysis were offered to the multivariable regression model. The strength of association was calculated using odds ratio at 95% CI.  $P < 0.05$  was considered as statistically significant.

### 2.6. Ethical approval

Ethical approval was obtained from our University research ethical review committee and it was in accordance with the ethical standards of our institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

## 3. Result

### 3.1. Socio-demographic characteristics of the study participants

Among 706 HIV positive patients attending ART, 135 (84.4%) was included in this study and the percentage of male and female attendees was 43.7% and 56.3%, respectively. Regardless of the disease condition, the mean age of the participants was (33.83 ± 11.11) years (Table 1).

#### 3.1.1. Seroprevalence of *T. gondii* infection

The overall seroprevalence of *T. gondii* infection in this study was 80.7% (109/135, CI: 0.740–0.874). Seroprevalence of *T. gondii* in relation to different socio-demographic factors is presented in Table 1.

#### 3.1.2. Risk factors associated with *T. gondii* infection

The bivariate logistic regression analysis showed a significant association between seropositivity to anti-*T. gondii* antibodies and presence of cat, raw meat consumption, knowledge about *T. gondii* and sex (Table 1). In multivariate logistic regression analysis, factors such as knowledge about toxoplasmosis, sex

**Table 1**

Distribution of *T. gondii* infection along with demographic characteristics of HIV patients ( $n = 135$ ).

Risk factors	HIV patients		
	Total	–ve (n, %)	+ve (n, %)
Age (years)			
15–24	16	4 (25.0)	12 (75.0)
25–36	74	17 (22.9)	57 (77.1)
36–48	27	6 (22.2)	21 (77.8)
>48	18	3 (16.7)	15 (83.3)
Educational status			
Illiterate	39	5 (12.8)	34 (87.2)
Literate	96	21 (19.3)	75 (78.1)
Sex			
Male	59	19 (32.2)	40 (67.7)
Female	76	7 (9.21)	69 (90.8)
Place residence			
Rural	76	11 (14.5)	65 (85.5)
Urban	59	15 (25.4)	44 (74.6)
Raw meat eating			
Yes	82	8 (9.8)	74 (90.2)
No	53	18 (34.0)	35 (66.0)
Owing cat			
Yes	65	7 (10.8)	58 (89.2)
No	26	19 (73.1)	7 (26.9)
Hand wash			
Yes	50	8 (16.0)	42 (84.0)
No	85	18 (21.2)	67 (78.8)
Raw vegetable			
Yes	69	13 (18.8)	56 (81.2)
No	66	13 (19.7)	53 (80.3)
Knowledge about <i>T. gondii</i>			
Yes	46	3 (6.5)	43 (93.5)
No	89	23 (25.8)	66 (74.2)
Raw milk consumption			
Yes	45	7 (15.6)	38 (84.4)
No	90	19 (21.1)	71 (78.9)
Religion			
Muslim	83	14 (16.9)	69 (83.1)
Orthodox	41	10 (24.4)	31 (75.6)
Protestant	11	2 (18.2)	9 (81.8)

and consumption of raw meat were significantly associated with seropositivity (Table 2).

Considering different age groups, the prevalence of *T. gondii* infection among age groups greater than 48 years was the highest. The prevalence in age category 15–24, 25–36, 37–48 and above 48 were 75% ( $CI$ : 0.279–32.209), 77.1% ( $CI$ : 0.259–4.129), 77.8% ( $CI$ : 0.079–1.472) and 83.3% ( $CI$ : 0.279–32.209),

**Table 2**

Multivariate analysis of independent predictor of *T. gondii* infection among HIV patients ( $n = 135$ ).

Risk factors	OR	95% CI	P
Presence of cat			
Yes	1.194	0.376–3.793	0.764
No	1		
Knowledge about toxoplasmosis			
No	5.225	1.382–19.762	0.015
Yes	1		
Sex			
Female	4.023	1.447–11.185	0.008
Male	1		
Consumption of raw meat			
Yes	3.514	1.167–10.581	0.025
No	1		

respectively and level of infection was observed to increase with age. However, there was no statistically significance association ( $P > 0.05$ ) observed between seropositivity and the different age groups. Moreover, there was no statistically significant association ( $P > 0.05$ ) between seroprevalence of *Toxoplasma* infection and level of education, hand washing, residence, religions, raw vegetable and milk consumption (Table 3).

It was observed that 48.1% of the participants had domestic cat at their home. Among HIV patients owning cat, 89.2% ( $CI$ : 1.200–7.940) were seropositive for *Toxoplasma* infection. Assessment of raw meat eating habit revealed that majority (60.7%) had the experience of eating raw meat. Among those with raw meat eating habit, the seroprevalence of *T. gondii* infection was 89.2% ( $CI$ : 1.887–11.995). In addition, knowledge about toxoplasmosis has strong association with *T. gondii* seroprevalence and the percentage of participants who had no knowhow about toxoplasmosis was 65.9%. Among those who has no knowledge about *T. gondii*, 93.5% ( $CI$ : 1.413–17.661) was seropositive. Regarding sex of participants, 56.4% were female attending HIV anti-retroviral therapy. The prevalence of anti-*T. gondii* seropositivity was significantly higher in females than males ( $P = 0.001$ ).

**Table 3**

Risk factors associated with *T. gondii* infection among HIV patients ( $n = 135$ ).

Risk factors	Seroprevalence		95% CI for EXP (B)		P
	–ve (n, %)	+ve (n, %)	OR	95% CI Lower Upper	
Age (years)					
15–24	4 (25.0)	12 (75.0)	1		
25–36	17 (22.9)	57 (77.1)	1.033	0.259 4.129	0.963
37–48	6 (22.2)	21 (77.8)	0.340	0.079 1.472	0.149
>48	3 (16.7)	15 (83.3)	3.000	0.279 32.209	0.364
Educational status					
Illiterate	5 (12.8)	34 (87.2)	1		
Literate	21 (19.3)	75 (78.1)	1.904	0.662 5.474	0.232
Sex					
Male	19 (32.2)	40 (67.7)	1		
Female	7 (9.21)	69 (90.8)	4.682	1.811 12.108	0.001
Place residence					
Rural	11 (14.5)	65 (85.5)	0.496	0.209 1.181	0.113
Urban	15 (25.4)	44 (74.6)	1		
Raw meat eating					
Yes	8 (9.8)	74 (90.2)	4.757	1.887 11.995	0.001
No	18 (34.0)	35 (66.0)	1		
Presence of cat at home					
Yes	7 (10.8)	58 (89.2)	3.087	1.200 7.940	0.019
No	19 (73.1)	7 (26.9)	1		
Hand wash					
Yes	8 (16.0)	42 (84.0)	0.709	0.283 1.775	0.463
No	25 (33.8)	49 (66.2)	1		
Raw vegetable					
Yes	13 (18.8)	56 (81.2)	0.946	0.402 2.227	0.900
No	13 (19.7)	53 (80.3)	1		
Raw milk consumption					
Yes	7 (15.6)	38 (84.4)	0.688	0.266 1.1783	0.442
No	19 (21.1)	71 (78.9)	1		
Knowledge about <i>T. gondii</i>					
Yes	3 (6.5)	43 (93.5)	1		
No	23 (25.8)	66 (74.2)	4.995	1.413 7.661	0.013
Religion					
Muslim	14 (16.9)	69 (83.1)	0.913	0.178 4.690	0.913
Orthodox	10 (24.4)	31 (75.6)	0.629	0.252 1.571	0.321
Protestant	2 (18.2)	9 (81.8)	1		

+ve: positive, –ve: negative.

#### 4. Discussion

*Toxoplasma* is an opportunistic parasite with grave public health impact in immuno compromised individuals. Acute infection of *T. gondii* results in IgM anti-*T. gondii* antibody response followed by enduring solid immunity IgG anti-*T. gondii* antibody which indicate chronic infection. Chronic infection of *T. gondii* develops encephalitis in HIV positive patients [20] especially in those individuals with CD4<sup>+</sup> T lymphocytes count level descend below 100 cells/ $\mu$ L [21]. Our study showed that *T. gondii* antibody in HIV positive individuals attending ART was 80.7%. This seroprevalence rate was high and is in agreement with previous studies conducted in different agro-ecological zones of Ethiopia [16–18,22].

The higher seroprevalence Esrates documented in this study might be because of individuals close interaction with cat as a pet animal, low level of awareness about means and route of transmission and poor hygienic conditions. However, the variation in seropositivity across different regions might be attributed to rate of reactivation of latent infection tissue toxoplasmosis when reduced cellular immunity in HIV patients (Zhou *et al.*, 2011) [23] and other socio-demographic factors such as, age of the study participants, type of test method used for detection of antibodies, feeding habit and living standard of individuals are some of the factors responsible for the variability of seroprevalence of toxoplasmosis in different agro-ecological zones across the globe. For instance, 19.9% in Malaysia (Brandon-Mong *et al.*, 2015) [24], 28.5% in woman in Democratic republic of Congo, 64.8% in western Romania (Olariu *et al.*, 2015) [25], 18.2% Southern Iran [26], 46% in Mozambique [27], 38.7% in Nigeria [28] and 54% in Uganda [29].

The seroprevalence of *T. gondii* antibodies in pre-ART as described also previously by different researchers [16,17] was higher than post-ART of this study. This could be possibly due to convalescing the immune status of patients, thus minimizing the incidence of opportunistic infections [30].

It has been documented that in immune-compromised individuals a previously acquired latent infection can lead to reactivation of toxoplasmosis causing severe encephalitis in about 40% of HIV patients and 10%–30% mortality was reported [31]. In view of this fact, almost all seroprevalence studies conducted so far including the present study, mainly focused on measuring the magnitude of antibody against latent infection and yet encephalitis is the most important sequel. This emphasizes on the need for further study on prevalence of encephalitis in this area and elsewhere for proper management of the cases at health centers.

In this study, latent toxoplasmosis seroprevalence was found to increase with age and the difference was not statistically significant. This finding is in agreement with reports [1,16,32]. This might be due to increased risk of exposure to infection with increasing age. In contrast, the report of [33] indicates significant association between seropositivity and age of patients.

The common traditional taboo of Ethiopia is raw/under cooked meat consumption accounts four times more likely seropositivity for toxoplasmosis. This finding is consistent with the reports of [16,17,22,34]. However, Muluye *et al.* [34] reported the absence of statistically significant association between seroprevalence of *T. gondii* infection and the habit of raw or under cooked meat consumption.

This inconsistency of raw meat consumption in predicting the seroprevalence of *T. gondii* in individuals depends on seroprevalence of *T. gondii* in meat producing animals which is 70.48% in sheep, 58.3% in goat in central Ethiopia [35] and 3.6% in cattle in Tanzania [36], 64% in chicken in Ghana [37]. Furthermore, the area is warmer and humid environment which is favorable climate for survival of the oocyst to maintain its lifecycle. As a result of this fact there is a possible contamination of the environment with *T. gondii* oocyst excreted by house or street cats available in the area. Our study revealed that contaminated environment with cat excreted oocyst is three times more likely for *T. gondii* antibodies seropositivity and this finding is in agreement with previous report of [17] and in contrast, with the finding of Yesuf and Melese [18] showed no association of presence of cat with *T. gondii* seropositivity.

Preponderance of the study participants had no knowledge about toxoplasmosis which is one of the independent predictor for the seroprevalence of *T. gondii*. Even though some of the participants had knowledge about the parasite, they did not know the potential predisposing factors and role of cat in the epidemiology of the toxoplasmosis. Our study also revealed that the odds of *T. gondii* seropositivity were four times higher in female in contrast with previous finding (Shimelis *et al.*, 2009). The study area is well known for its cash crop type of Agriculture whereby gardening and cropping is conducted by women which is the main contributing factor for the high seroprevalence of the disease in women. The ratio of female to male currently attending ART at Agaro health center is 1.8:1 this might be another attributing factor for the activation of latent toxoplasmosis higher in women.

The overall seroprevalence of *T. gondii* infection antibodies among HIV patients was high and some potential independent risk factors are significantly associated with seropositivity of *T. gondii* infection. Furthermore, raw meat consumption is a potential source of the parasites and the recommendation should be to avoid raw meat consumption to prevent possible ingestion of viable cysts in meat which modulate immune system conversely decrease CD4<sup>+</sup> cells leading to activation of the latent toxoplasmosis.

Considering this fact, we recommend toxoplasmosis chemoprophylaxis in all HIV positive individuals to prevent encephalitis. Other hygienic procedures such as hand washing after gardening/farming to minimize the contamination of oocyst need to be included in counseling package of patients. Knowledge, attitude and practice about toxoplasmosis were low and thus, education of the public is needed to create good awareness about *T. gondii* transmission and risk factors. Moreover, it is highly advisable for health officers in charge to include routine serological screening test for determination of anti-*T. gondii* antibodies among HIV infected individuals pre-ART.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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